Constructing AOPs for Developmental Toxicities

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ILS, Inc./NICEATM
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The views expressed in this presentation are those of the author[s] and do not necessarily reflect the views or policies of the U.S. EPA or any government organization.

- move away from animal testing to high-throughput and *in vitro* assays to understand how chemicals perturb cellular functions
- establish linkage between molecular and cellular perturbations *in vitro* and adverse outcomes *in vivo* (*adverse outcome pathways*)
- HTS: broader coverage of chemical landscape, faster and cheaper assessment of biological activity, and much fewer animals (*3 R’s*)
HTS Paradigm

- High-throughput screening
  fast screening of chemical libraries
  SCALE

- Vast data on many chemicals

- High-content screening
  detailed imaging, arrays
  PARALLELISM

Challenge: Interpreting all of the data

- Systems Modeling

- Text-mining, data-mining
- Bioinformatics, pathways
- Networks, systems
Toxicity Forecaster (ToxCast) & Tox21

- **ToxCast**: U.S. EPA research program profiling over 2,000 chemicals across >700 *in vitro* assays. [http://www.epa.gov/ncct/toxcast/](http://www.epa.gov/ncct/toxcast/)

  - **Phase-I**: 309 data-rich chemicals (primarily pesticides) having over 30 years of traditional animal studies valued at $2B; *in vitro* signatures defined by how well they can predict toxicity in the animal studies.

  - **Phase-II**: 776 chemicals from a broad range of sources (e.g., industrial and consumer products, food additives, failed drugs) to extend and apply first generation predictive models of toxicity.

  - **Phase-III**: 1000 chemicals in a subset of assays, follow-up targeted testing

- **Tox21**: partnership of NCATS (National Center for Advancing Translational Science), U.S. EPA (Computational Toxicology), NIEHS (National Toxicology Program), and FDA to screen 10,000 compounds in 50 assays/year.
ToxCastDB: 700+ HTS Assays

**Assay Provider**
- ACEA
- Apredica
- Attagene
- BioSeek
- NCGC/Tox21
- NHEERL MESC
- NHEERL NeuroTox
- NHEERL Zebrafish
- NovaScreen
- Odyssey Thera
- Vala

**Species**
- Human
- Rat
- Mouse
- Zebrafish
- Sheep
- Boar
- Rabbit
- Cattle
- Guinea pig

**Cell Format**
- Cell free
- Cell lines
- Primary cells
- Complex cultures
- Free-living organisms

**Readout Type**
- Single
- Multiplexed
- Multiparametric

**Biological Response**
- cell proliferation and death
- cell differentiation
- mitochondrial depolarization
- protein stabilization
- oxidative phosphorylation
- reporter gene activation
- gene expression (qNPA)
- receptor activity
- receptor binding

**Tissue Source**
- Lung
- Liver
- Skin
- Cervix
- Uterus
- Intestinal
- Bladder
- Pancreas
- Inflammatory

**Target Family**
- Response Element
- Transporter
- Cytokines
- Kinases
- Nuclear Receptor
- CYP450 / ADME
- Cholinesterase
- Phosphatases
- Proteases
- XME metabolism
- GPCRs
- Ion Channels

**Assay Design**
- viability reporter
- morphology reporter
- conformation reporter
- enzyme reporter
- membrane potential reporter
- binding reporter
- inducible reporter

**Detection Technology**
- qNPA and ELISA
- Fluorescence & Luminescence
- Alamar Blue Reduction
- Arrasyscan / Microscopy
- Reporter gene activation
- Spectrophotometry
- Radioactivity
- HPLC and HPEC
- TR-FRET

[http://actor.epa.gov/actor/faces/ToxCastDB]
ToxRefDB: *in vivo* Study Data

- ToxRefDB holds *in vivo* endpoint data from animal toxicology studies (DERs, NTP, open literature, and pharma)

- currently at 1049 chemicals with 5567 studies (2147 Phase I, 1682 Phase II, 481 e1k, 1047 not in ToxCast)

**CHRONIC/CANCER**
Martin et al. (2009) Environ Hlth Persp

**MULTIGENERATIONAL REPRODUCTIVE**
Martin et al. (2009) Toxicol Sci

**PRENATAL DEVELOPMENTAL**
Knudsen et al. (2009) Reprod Toxicol

http://actor.epa.gov/toxrefdb

**SOURCE:** Matt Martin, NCCT
Predicting adverse outcomes from in vitro data

Endpoints *(in vivo)*
ToxRefDB

Endpoints *(in vitro)*

Chemicals
ToxCast (1060)
e1K (1860)
Tox21 (8193)

Effects *(in vitro)*
ToxCastDB

LEL

positive

associated w/ effect

no LEL

negative

associated w/ no effect
1st Generation Predictive Models

- **Predictive models: endpoints**
  - liver tumors: Judson et al. 2010, Env Hlth Persp 118: 485-492
  - dev tox and hESC: Kleinstreuer et al. 2011, Tox App Pharm 257(1):111-21
  - zebrafish development: Sipes et al. 2011, Birth Defects Res C 93: 256-267

- **Predictive models: modes of action**
  - endocrine disruption: Reif et al. 2010, Env Hlth Persp 118: 1714-1720
  - angiogenesis: Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603

2nd gen. model: Adverse Outcome Pathway Framework
Knudsen & Kleinstreuer 2012, BDRC 93(4):312-23
Vascular Developmental Processes

- endothelial proliferation & cell migration
  - growth factors
  - chemokine signaling

- extracellular matrix degradation
  - plasminogen activating system
  - matrix metalloproteinases

- neovascular stabilization
  - Ang/Tie2 signaling
  - vascular remodeling

**eLibrary AngioKB:**
~100 distinct ToxCast assay targets map to key systems in vascular development

ToxCast Assay Target

Adapted from Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603
Provide overview of the literature
- connections between chemicals, protein targets and biological effects
- delivered in a spreadsheet with hyperlinks to PubMed

Advantages for AOP elucidation
- organize & navigate information into weight-of-evidence schema
- ‘stumble-upon’ effect (helps avoid looking under the lamp-post)

Identify chemicals, proteins, and effects co-annotated with vascular keywords

SOURCE: Nancy Baker, Lockheed-Martin
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<th>Protein</th>
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<th>Wound</th>
<th>Cancer</th>
<th>NeoVasc Phys</th>
<th>NeoVasc Path</th>
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<td>The COX-2/PGF2alpha pathway: key roles in the hallmarks of cancer and adaptation to the tum</td>
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<td>Platelet-activating factor in anesthetic liver and hepatic cell carcinoma</td>
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<td>The proangiogenic phenotype of tumor-derived endothelial cells is reverted by the overex</td>
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<td>Effects of deletion of the protooncogene on ovarian gene expression</td>
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<td>Chondrocyte-specific modulation of Cyp27b1 expression supports a role for local synthesis</td>
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<td>VEGF-induced HUVEC migration and proliferation are decreased by PDE2 and PDE4 inh</td>
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<td>Cyclic GMP-mediated macromolecular extraction from angiogenic microvesicles in vivo</td>
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<td>Nitric oxide enhances angiogenesis via the synthesis of vascular endothelial growth factor</td>
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<td>Functional recovery in aged and young rats after embolic stroke: treatment with a phospho</td>
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<td>Tocilizumab, a long-acting type 5 phosphodiesterase isozyme inhibitor, improves neurologi</td>
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<td>Differing mechanisms of cell-induced changes in capillary supply in the limbs of anterior</td>
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<td>Physicochemical characteristics of solute algogenic Akita and their pathogenic role in A</td>
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<td>3-Hydroxyacyl CoA Dehydrogenases</td>
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<td>15848588</td>
<td>2005</td>
<td>App is no barrier to muscle structural, biochemical and angiogenic adaptations to training</td>
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</tbody>
</table>
Gene Ontology (GO) and Mammalian Phenotype (MP) browsers of MGI database (http://www.informatics.jax.org/) for neovascularization:
- abnormal vasculogenesis [MP:0001622; 72 genotypes, 73 annotations]
- abnormal angiogenesis [MP:0000260; 610 genotypes, 894 annotations]

65 genes with roles in vasculogenesis or angiogenesis linked to ToxCast assays, 50 had evidence of abnormal embryonic vascular development in MGI.
Proposed AOP: Embryonic Vascular Disruption

- VDCs: 
  - Hypoxia (↓O2, ↑ROS)
    - HIF1α, AhR
  - Angiogenic switch
    - VEGF, FGF
    - Notch-Dll4 signals
  - Chemokine pathway
    - CCL2, CXCL10, Il-1, TNFα
  - ECM interactions
    - uPAR, PAI-1, MMPs, Intg
  - Vessel remodeling
    - Prolif., TGFβ, EphA/B, TIE2

- Angioblasts
  - ↓vasculogenesis
  - ↓blood islands

- Endothelial cells
  - ↓cytoskeletal cycle
  - ↓angiogenic sprouts

- Macrophage cells
  - ↓cell mobility
  - ↓growth factor release

- Mural cells
  - ↓cell recruitment
  - ↓vessel stabilization

- Placenta
  - Nutrient exchange
  - Altered physiology
  - Impaired blood flow

- Embryo-Fetus
  - Altered hemodynamics
  - Impaired growth
  - Dysmorphogenesis
  - Altered differentiation

- Newborn
  - Low birth weight
  - Functional deficit
  - Malformation
  - Lethality

- Population
  - Developmental health consequences

**Key**
- Established mechanistic linkage with quantitative or semi-quantitative data
- Predictive model linkages based on quantitative concentration-response data
- Plausible linkage with limited data
- Hypothetical linkage
- Empirical linkage based on quantitative exposure-response data
- Assay linked to ToxCast

ER

pVDC

ToxPi
Ranking by pVDC AOP score: 1060 ToxCast compounds

Thalidomide structural analogue
- disrupts angiogenesis

5HPP-33 (0.683)

Mitocide/insecticide
- mitochondrial respiratory chain

Pyridaben (0.667)

Herbicide/weed control
- acetohydroxyacid synthesis

Imazamox (0.02)

Toxicity Prioritization Index (ToxPi) for vascular disruption
**ToxCastDB**

http://actor.epa.gov/actor/faces/ToxCastDB/DataCollection.jsp

**AC50** concentration producing a 50% change

**LEC** lowest effect concentration
Test 36 ToxCast Phase I & II chemicals
(wide range of pVDC scores)

A. Toxicity Prioritization Index (ToxPi) for vascular disruption based on ToxCast in vitro assays

B. CC3D Virtual tissue model

C. Eli Lily angiogenesis assay
   co-culture of human endothelial progenitor cells (ECFCs) with adipose-derived adult stem cells (ADSCs)

D. Quantitative zebrafish vascular toxicity assay

E. FICAM angiogenesis assay
Abstract

Vascular development is a complex process regulated by dynamic biological networks that vary in topology and state across different tissues and developmental stages. Signals regulating de novo blood vessel formation (vasculogenesis) and remodeling (angiogenesis) come from a variety of biological pathways linked to endothelial cell (EC) behavior, extracellular matrix (ECM) interactions, and cell-cell communications.
Validation of vascular disruption AOP by orthogonal assays: *in vitro, in silico, and in situ*

**In vitro (HUVEC)**
Adapted from Noguchi et al. 2005, Bioorg Med Chem Lett.

**In silico (virtual tissue)**
Adapted from Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996

**In situ (Aortic explant)**
Source: E. Carney & R. Ellis-Hutchings, Dow Chemical Co.

5HPP-33 exposure disrupts angiogenesis *in vitro, in silico, and in situ*
Virtual tissues predictions: Environmental pVDCs

ToxCast prediction: Pyridaben

Virtual Tissue model: Pyridaben

In vitro qualification: Pyridaben

AC50 = 0.0056 uM

Cytotox = 5.0 uM

ToxCast prediction: Imazamox

Virtual Tissue model: Imazamox

In vitro qualification: Imazamox

AC50 = 8109 uM

stimulatory

SOURCE: T. Heinonen and R. Sarkanen, FICAM
Human Cell Based Angiogenesis Assay

- 7 ToxCast Phase I test compounds
- 5 predicted positives (pVDCs)
- 2 predicted negatives (non-pVDCs)
- Concentration-response effects on vessel formation in angiogenesis assay?

[Adapted from Sarkanen et al. 2011]
<table>
<thead>
<tr>
<th>Test Chemicals &amp; pVDC ToxPi</th>
<th>ToxRefDB Rat dLEL (mg/kg)</th>
<th>ToxRefDB Rabbit dLEL (mg/kg)</th>
<th>ToxCast pVDC prediction</th>
<th>Virtual tissues model prediction</th>
<th>Angiogenic Inhibition 50% ↓ (µM)</th>
<th>Initial Cytotoxicity 20% ↓ (µM)</th>
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<td>+</td>
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A Quantitative Vascular AOP model: Zebrafish embryogenesis

- a biologically complex system to study vascular developmental toxicity
- conserved pathways
  - 75% of genes have human homologs
- embryo is transparent
  - amenable to quantitative imaging
- transgenic reporter lines
  - map vasculature across space-time
- rapid and scalable platform
  - amenable to automation and HTS

SOURCE: Tamara Tal, EPA/NHEERL-ISTD
SUMMARY

- Constructing quantitative AOPs yields hypothesis generation and testing of MIEs and cellular interactions that may lead to developmental toxicity.

- Developing and identifying resources for high quality *in vivo* data allows linking perturbations to phenotypic endpoints.
  - MGI DB, eLibrary literature curation, ontology construction, etc.

- AOP validation is facilitated via HTS data, orthogonal assays, small model organisms and other scientifically relevant information.

- Validated AOPs will enable chemical prioritization and high throughput risk assessments.
Scientific Workshop

Adverse Outcome Pathways in Environmental Regulation

September 3-5, 2014
Natcher Center
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Questions?